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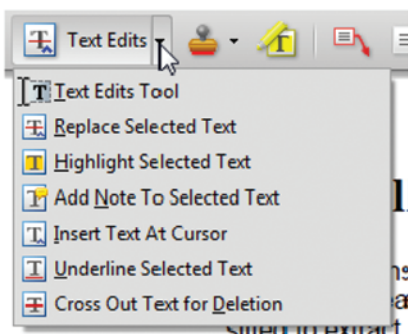
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The pandemic pathogen of amphibians, *Batrachochytrium dendrobatidis* (Phylum Chytridiomycota), in Italy

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Abstract

Worldwide amphibian declines and species losses are global problems and emerging infectious diseases have been identified as one of the major threats. The worst of these is chytridiomycosis, an amphibian disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*). Here we review what is known of the distribution of *Bd* and chytridiomycosis in Italy. We critically summarize the evidence in support of the hypothesis that *Bd* is an invasive pathogen in Italy. Last we provide recommendations for immediate research needs, both for basic science and applied conservation.

Keywords: *Amphibian conservation, Batrachochytrium dendrobatidis, chytridiomycosis, infectious disease, Italy*

Introduction

Amphibian species are threatened worldwide, with approximately one third of the 6000+ living and described species in decline and more than one hundred of these potentially extinct since the 1980s (Stuart et al. 2004). Major threats that have been identified include habitat destruction and alteration (Alford & Richards 1999; Cushman 2006), environmental contaminants such as UV-B irradiation and chemical pollutants (Blaustein et al. 2001, 2003), the global trade in amphibians (Schlaepfer et al. 2005), the introduction of alien species (Kats & Ferrer 2003) and emerging infectious diseases (Carey et al. 1999; Daszak et al. 1999). The chytridiomycete fungus *Batrachochytrium dendrobatidis* (henceforth *Bd*; Berger et al. 1998; Longcore et al. 1999) is the cause of chytridiomycosis, which is considered the infectious disease that poses the greatest threat to amphibian biodiversity. This is because *Bd* has been implicated in numerous mass mortalities and population declines and may be the cause of local extirpation and global extinction of multiple amphibian species (Daszak et al. 2000; Fisher et al. 2009b).

Bd is known to infect over 500 amphibian hosts and occurs on all continents where amphibians are found (Daszak et al. 2003; Rachowicz et al. 2006; Fisher et al. 2009b; <http://www.bd-maps>). The spatial distribution of *Bd* is highly heterogeneous (Walker et al. 2010; <http://www.bd-maps>) and the fungus is composed of multiple genetically distinct lineages that exhibit variable virulence and distributions (Fisher et al. 2009a; James et al. 2009; Farrer et al. 2011). In accordance with these findings, and due to its ability to cause declines of amphibian populations and species, *Bd* is listed as a notifiable pathogen by the World Organization for Animal Health (OIE 2008). However, the presence of *Bd* does not always translate into an immediate threat to a host: impacts of infection can vary from no or mild disease to severe disease, mass deaths and population decline (Daszak et al. 1999; Bosch et al. 2001; Lips et al. 2006, 2008; Kriger & Hero 2007; Bielby et al. 2008; Fisher et al. 2009b; Vredenburg et al. 2010; Walker et al. 2010).

Chytridiomycosis is commonly associated with cutaneous infection of metamorphosed individuals, while the infection of tadpoles is restricted to

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keratinized mouthparts and unlikely to result in death (Berger et al. 1998). Severe disease in metamorphosed animals can occur rapidly and has been associated with the disruption of cutaneous function and other physiological imbalances, including inhibition of electrolyte transport across the epidermis, reduction of sodium and potassium concentrations in cell plasma and asystolic cardiac arrest (Voyles et al. 2007; Scott et al. 2010). The clinical signs of *Bd* infection are often absent, transient and/or nonspecific and therefore insufficient for diagnosis, but include lack of mobility and reflexive responses to external stimuli and orientation, skin shedding, deformed and depigmented tadpole mouthparts and, in some species, ulceration and necrosis of extremities (Densmore & Green 2007; Duffus & Cunningham 2010). Confirmed diagnosis of infection usually requires an initial molecular diagnostic followed by histological identification of the presence of immature stages and/or intracellular sporangia (Boyle et al. 2004; Hyatt et al. 2007).

Bd has been implicated in amphibian mortalities and declines beginning in the 1970s in the Americas and Australia (Skerratt et al. 2007 and references therein) but the two initial records for *Bd* infections in Europe occurred decades later. The first record of *Bd* in wild European amphibians comes from the late 1990s and describes chytridiomycosis-related mass mortalities in the Sierra de Guadarrama Mountains of Spain (Bosch et al. 2001). The first record in captivity describes infection of imported, wild-collected animals as well as captive-reared amphibians, both sampled in 1999 in Germany and Belgium (Mutschmann et al. 2000). Since then our knowledge regarding the distribution of infection in Europe and European hosts has increased substantially (Garner et al. 2005; Bosch & Martínez-Solano 2006; Garner et al. 2006; Bosch et al. 2007; Bovero et al. 2008b; Walker et al. 2008; Bielby et al. 2009; Garner et al. 2009b; Pasmans et al. 2010; Tobler & Schmidt 2010; Walker et al. 2010; Ohst et al. 2011; Sztatecsny & Glaser 2011; ~~www.bd-maps.eu/~~). *Bd* was detected for the first time in Italy in 2000 (as reported in Stagni et al. 2004), but little is known regarding the impact of *Bd* on Italy's highly diverse amphibian fauna. This is a concern, as some of Italy's amphibians have experienced rapid declines that are similar to those due to chytridiomycosis in the New World and Australia, and two Italian endemics have experienced significant lethal chytridiomycosis (Stagni et al. 2004; Bielby et al. 2009). In this review we summarize the state of knowledge regarding the distribution of *Bd* in Italy and the impact of chytridiomycosis on Italian amphibians. The specific objectives of the review are: (1) to summarize what is known of the

distribution of *Bd* in Italy and the host species that are infected; (2) to hypothesize as to the origin of the pathogen in Italy, and (3) to provide recommendations for further research, both basic and applied, regarding *Bd* in Italy.

The known distribution of *Batrachochytrium dendrobatidis* in Italy and Italian amphibians

In Italy *Bd* has been detected in six species of anurans: *Bombina pachypus*, *Discoglossus sardus*, *Lithobates catesbeianus*, *Pelophylax lessonae*, *Pelophylax kl. esculentus* [note: species identification in Simoncelli et al. (2005) may be inaccurate; water frog host species may actually be *P. bergeri* and *P. kl. hispanicus*] and *Rana latastei*, and one species of caudate, *Euproctus platycephalus* (Table I; Figure 1), representing both endemic and introduced species.

The first record of *Bd* in Italy is a report of lethal chytridiomycosis affecting endemic and endangered Apennine yellow-bellied toads (*B. pachypus*) in the province of Bologna (Stagni et al. 2002, 2004). In 2000 and 2001 juveniles that were part of a head-starting project (Pritchard 1979) experienced severe mortality and exhibited known clinical signs of chytridiomycosis and unusual behaviour, including skin shedding and anorexia, as well as the tendency to leave water and hyperaemia of the digits (Stagni et al. 2004). Infection with *Bd* was shown retrospectively using both molecular and histological analyses. Subsequent searches at the locations where animals that experienced lethal chytridiomycosis had been collected revealed little evidence of successful recruitment in the years when mortality in captivity was observed. No direct evidence of lethal chytridiomycosis or infection with *Bd* in the wild was reported for these or any of the locations involved in the head-starting project (Stagni et al. 2004).

The majority of other studies of *Bd* in Italy are even more limited. Most reports involve extremely small sample sizes haphazardly collected, only provide single or a few data points showing presence of infection, and are inadequate for more general spatial analyses or comparisons of prevalence amongst hosts (Garner et al. 2006; Adams et al. 2008; Bovero et al. 2008b; Federici et al. 2008; Ficetola et al. 2011). Somewhat more robust and targeted sampling by Simoncelli et al. (2005) detected infection with *Bd* in the nonhybrid member of a *Pelophylax* spp. hybridogenetic complex, with no evidence of disease. The low number of infections detected in their study precludes making any statistical comparison of prevalence amongst hybrid and nonhybrid species; however, infection of water frogs without evidence of disease or mortality is in broad agreement with

Table 1. Species *Batrachochytrium dendrobatidis* (Bd)-positive in Italy with information on International Union for Conservation of Nature (IUCN) category (LC = Least Concern; VU = Vulnerable; E = Endangered), locality, number of analyzed samples, number of positive samples, method of detection used, detected symptoms and references.

Species	IUCN	Italian Region	No. sampled	No. positive	Method of detection	Consequences of infection	Source
<i>Discoglossus sardus</i>	LC	Limbara Mountain (OT), Sardinia	72	33	PCR/ histology	Tips of the digit damaged, death, moribund animals	Bovero et al. 2008a; Bielby et al. 2009
<i>Lithobates catesbeianus</i>	LC	near Turin, Piedmont southeastern Piedmont	37 4	1 3	PCR PCR	Not indicated Healthy	Garner et al. 2006 Adams et al. 2008
<i>Pelophylax lessonae</i> (<i>P. bergeri</i>)	LC	Trasimeno Lake District, Umbria	80	4	PCR/ histology/ Immunohisto- chemistry	Skin lesion in abdominal, pelvic, femoral regions and feets	Simoncelli et al. 2005
		southeastern Piedmont	9	1	PCR	Healthy	Adams et al. 2008
<i>Pelophylax kl. esculentus</i>	LC	Bosco della Mesola, Ferrara, Emilia-Romagna	3	1	PCR	Healthy	Ficetola et al. 2011
		Pianalto di Poirino, Turin, Piedmont	45	13	PCR	Healthy	Federici et al. 2008
<i>Rana latastei</i>	VU	Western Po plane (unknown locality)	Not indicated	Not indicated	PCR	Not indicated	Garner et al. 2004
<i>Bombina pachypus</i>	E	Bologna, Emilia-Romagna	110	50	PCR/ histology	Scaling off of the skin, anorexia, numbness, tendency to leave water, hyperanemia of the fingers, death (captivity)	Stagni et al. 2002, 2004
<i>Euproctus platycephalus</i>	E	Sette Fratelli Mountain, (CA), Sardinia	5	3	PCR	Tips of the digit damaged	Bovero et al. 2008b
		Limbara Mountain (OT), Supramonte di Urzulei (NU), Gennargentu (NU), Sette Fratelli Mountain (CA), Sardinia	303	36	PCR	Tips of the digit damaged	Bovero et al. 2008a

PCR, polymerase chain reaction.

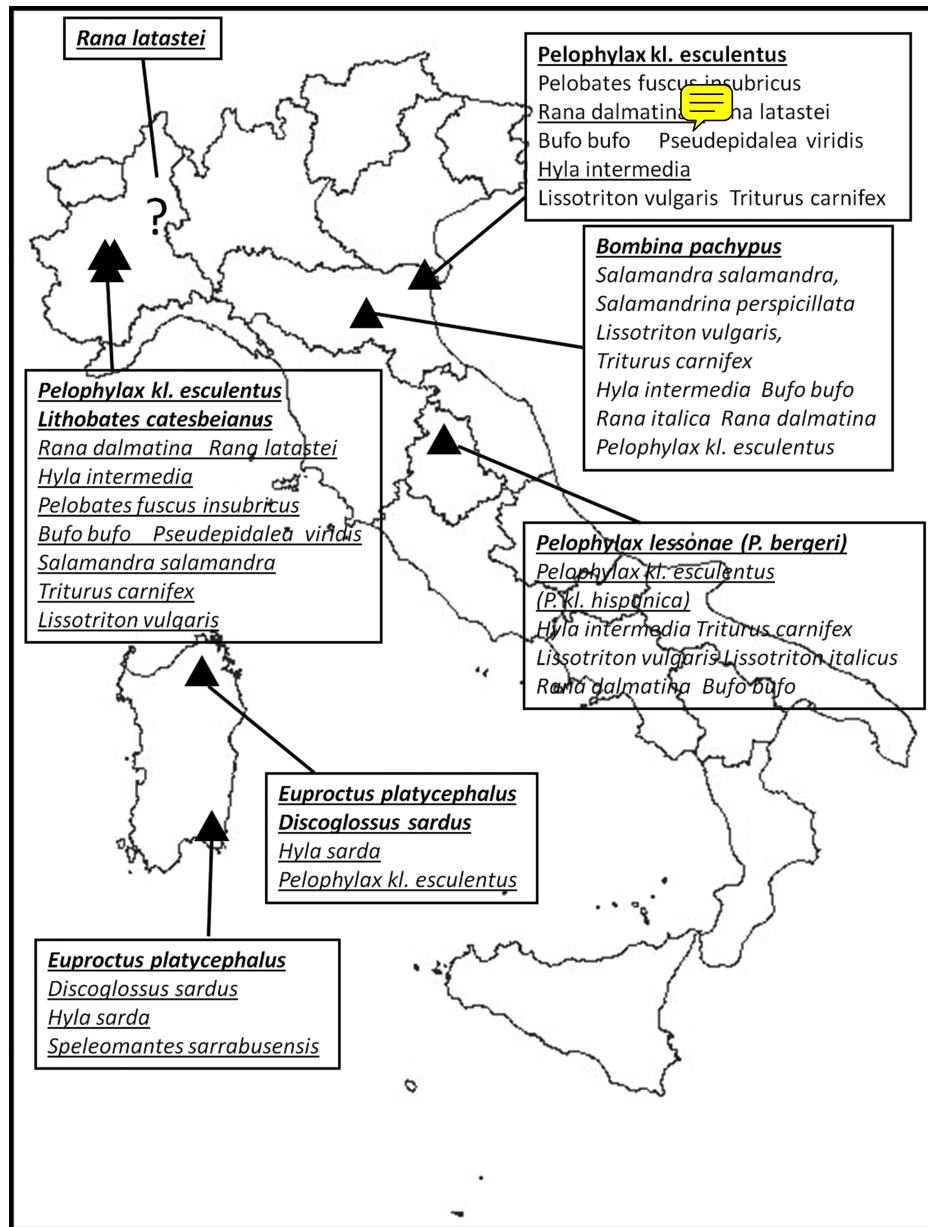


Figure 1. Map of Italy. Triangles represent *Batrachochytrium dendrobatidis* (*Bd*)-positive sites with the indication of amphibian species of the area (Andreone & Sindaco 1998; Mazzotti et al. 1999, 2007; Carletti & Spilinga 2006; Bovero et al. 2008a). Species positive to *Bd* analyses are underlined and in bold; Species negative to *Bd* analyses are underlined; the other species in the area were not analysed.

existing data from other parts of Europe (Ohst et al. 2011; www.bd-maps.eu/). The only published evidence of lethal chytridiomycosis in Italian amphibians outside of captivity remains the study by Bielby et al. (2009). Unusual mortality of *D. sardus* at three locations in northern Sardinia was associated with *Bd* infections detected in dead animals or infection of conspecifics at the location where mortality was previously observed. The species persists at all locations where mortality was observed but, based on limited field data, local frog and tadpole abundances

are now low, and evidence of breeding at these sites is extremely limited when compared to the density of clutches, tadpoles and metamorphs observed at some breeding sites on the island (*Zirichiltaggi* – SWC, unpubl. data). Robust breeding assemblages of *Hyla sarda* or *Euproctus platycephalus* occur at two of these locations, and co-occurring *E. platycephalus* also exhibit persistent, interannual infections without any observed evidence of unusual mortality (Bovero et al. 2008a, 2008b; *Zirichiltaggi* – SWC, unpubl. data).

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The data available from these few published studies make a definitive statement regarding distribution of *Bd* in Italy impossible. At a minimum, *Bd* occurs in four provinces, distributed across half of Italy's continental land mass and including one major island (Figure 1). Only 17 of Italy's 49 amphibian species have been tested for infection, and in many cases species-level sampling efforts involve single sites and insufficient replication to accept negative results as a reliable indication of disease status. Some of the species that did not test positive in Italy are infected in other parts of Europe (Garner et al. 2009b; Ohst et al. 2011; Sztatecsny & Glaser 2011). Together these suggest that existing data substantially underestimate the actual distribution of *Bd* in Italy. Notably, the study by Bielby et al. (2009) is the first evidence in Europe of significant adult anuran mortality linked with possible declines. Other published studies of lethal chytridiomycosis in European anurans report mortality primarily in near- or recently metamorphosed juveniles (Bosch et al. 2001; Bosch & Martínez-Solano 2006; Garner et al. 2009b; Pasmans et al. 2010; Tobler & Schmidt 2010; Walker et al. 2010). Mortality and decline of *Salamandra salamandra* involved some adult mortality, but even in this case the majority of dead animals observed by Bosch & Martínez-Solano (2006) were near- or recently metamorphosed juveniles.

What can be said about the origin of *Bd* in Italy?

The debate regarding the global emergence of *Bd* scales to the national level. Is *Bd* a newly emerged pathogen recently introduced to areas by animal (including human) or other vectors where it is causing population declines (Novel Pathogen Hypothesis), or is *Bd* an endemic parasite and recent disease emergences are attributable to increased virulence caused by (human-mediated) ecological and environmental changes (Endemic Pathogen Hypothesis; Rachowicz et al. 2005; Skerratt et al. 2007)? Some authors argue that both mechanisms contribute to the existing distribution of presence, prevalence and disease (Goka et al. 2009; Walker et al. 2010) but the fundamental question regarding introduction remains valid, as efforts to mitigate disease may differ based on whether a pathogen is introduced or not. Evidence in support of introduction includes the presence of infection in commercially traded amphibian species both at source and in invasive populations (Mazzoni et al. 2003; Weldon et al. 2004; Fisher & Garner 2007). Africa is considered a potential source of the original *Bd* vector (*Xenopus* spp.; Weldon et al. 2004; Walker et al. 2008; Farrer

et al. 2011), as is North America (*Lithobates catesbeianus*; James et al. 2009), although the possibility of an Asiatic source of *Bd* is currently under debate (Fisher 2009; Goka et al. 2009). Italy has a relatively rich and long history of commercial amphibian species introductions, movement of regional species for food, trade and other purposes, and the establishment of introduced populations of both non-native and native species (Lanza 1983; Lanza & Ferri 1997; Lillo et al. 2005; Ficetola & Scali 2010). Introduced populations of potential hosts do occur in all four provinces where *Bd* is known to occur, and at least one introduced species, the bullfrog *Lithobates catesbeianus*, does carry infections in the wild in Italy (Garner et al. 2006).

Recent comparative population genomics analyses have shown that *Bd* comprises at least three divergent lineages, one of which is recently evolved, globally distributed, highly virulent and associated with mass mortality events (Farrer et al. 2011). The occurrence of a newly globalized, virulent form (referred to as *Bd*GPL in Farrer et al. 2011) strongly implicates global trade mediating intercontinental spread. Interestingly, the only continent where all three lineages were detected is Europe, and a general assumption of molecular epidemiology is that the source of an emerging pathogen with a rapidly expanding range should exhibit the greatest amount of phylogenetic variability. Higher genetic variability in Europe requires confirmation through extended sampling outside of Europe, as the majority of isolates used in the study were European. No Italian isolates were included in the study by Farrer et al. (2011), but several isolates of *Bd* have been collected from Sardinia (*Zirichiltaggi* – SWC, unpubl. data), all from *D. sardus*. Three isolates from two drainages located within a disease cluster on Sardinia have been sequenced and fall within the more virulent *Bd*GPL clade (R. Farrer, D. Henk, M. Fisher, unpubl. data).

The presence of infected invasive amphibians, along with the localized occurrence of the global panzootic lineage *Bd*GPL on the island of Sardinia, are indicative of recent introduction to Italy through amphibian trade. More careful review of the existing evidence shows that this conclusion is not yet fully justified. Infected invasive amphibians are only reported for the Po Plain (Garner et al. 2006) and all efforts to detect *Bd* infection of invasive species on Sardinia (e.g. water frogs; Li Vigni et al. 2011) have returned negative results (Bovero et al. 2008a). Infection of native Sardinian species occurs predominantly in mountain areas which are subject to sport fish introductions (Orrù et al. 2010), a proposed route for *Bd* introduction outside the amphibian trade (Johnson & Speare 2005), but a range of human

activities occur in areas where infection has been detected on the island. *BdGPL* is widespread across Europe and is expanding its range in some locations in a wave-like fashion, a pattern that can be explained by natural movement of infected hosts (Walker et al. 2010; Farrer et al. 2011). It is reasonable to hypothesize that at least part of the Italian distribution of the fungus is the result of introduction of some sort. Determining what part will require careful investigation of not only how introduced amphibians may have been responsible for the spread of *Bd* in Italy, but how all potential routes of disease introduction may have contributed. Furthermore, without structured *Bd* spatial data collected on a national scale and investigations of how environmental factors may correlate with infection and disease, *Bd* endemicity cannot be ruled out.

Is there cause for concern? Recommendations for further research

The Italian batrachofauna is composed of 49 different species, 21 of which are endemic along with six endemic subspecies. Three species are listed as endangered by the International Union for Conservation of Nature (IUCN); two of them, *E. platycephalus* (Romano et al. 2009) and *B. pachypus* (Andreone et al. 2009a), are known to be infected with *Bd* and the third, *Speleomantes supramontis* (Andreone et al. 2009b), occurs on Sardinia where *BdGPL* infects native amphibian species. All three species occupy small ranges, a trait that predisposes species to declines due to chytridiomycosis (Bielby et al. 2008). These facts alone are cause for concern and should act as a call for increased national and EU funding supporting research and, where warranted, conservation interventions. Regional investment in *Bd* research has been made, albeit rarely: screening for *Bd* infection of *E. platycephalus* carried out in 2010 by *Zirichiltaggi* – SWC was in part funded by Ente Foreste delle Sardegnia. While this project increased understanding and awareness of infection in a threatened Italian amphibian, it is only a small part of the regional and national efforts that will be required to gain relatively comprehensive insight into the distribution of *Bd* in Italy and where the risks to the Italian amphibians may lie.

Initial research efforts should first expand our understanding of the distribution of *Bd* in Italy, both geographically and in terms of host species range. This will require structured, statistically defensible sampling based on hypothesis testing. Studies have shown that *Bd* exhibits temporal as well as spatial variation and both prevalence and disease are influenced by environmental factors (Fisher et al. 2009b).

Substantial gains of insight into infection and disease dynamics have been achieved by sampling focal species and life history stages (Bosch et al. 2007; Kriger & Hero 2007; Briggs et al. 2010; Vredenburg et al. 2010; Walker et al. 2010). Most of these and other informative studies of *Bd* have involved site, species and life history stage-specific sample sizes exceeding 20 individuals or more. We recommend researchers take advantage of existing data regarding infection of European species, both native and introduced, and how prevalence may vary, to design their initial survey strategy. Sampling species that are known to be infected and exhibit higher prevalence increases the likelihood that infection will be detected in another survey, data will be amenable to statistical analyses, and comparisons are more easily made with other European study systems. We also strongly recommend that those intending to embark on *Bd* research first consult the epidemiological literature and develop a robust sampling strategy that is suitable for spatial epidemiological statistical analyses. The optimal strategy is to work with researchers experienced in spatial epidemiology, wildlife diseases and host/parasite dynamics. Continued opportunistic sampling will only add a few points to an already scant distribution map without helping us to understand if Italy's amphibian assemblages are under threat from this infectious disease. To our knowledge, several studies of *Bd* in Italy are currently ongoing (see Table II).

Inevitably, studies of some species will not be compatible with strict sampling designs because so many of Italy's amphibians are endemic and there are no preliminary data available regarding susceptibility to infection (data on congeners may exist, and be informative). Many of these species are also cryptic and difficult to sample in numbers appropriate for any quantitative biological study. Those intending to undertake *Bd* research on such species should expect to spend more time and expend more effort before seeing academic returns on their investment even though their efforts may contribute to conservation. Alternatively, research on a difficult-to-study amphibian species of conservation concern may be coupled with a parallel study of another species that is easier to sample meaningfully and has the strong potential to interact with the threatened species. In this way robust, highly publishable science perhaps need not be sacrificed for the sake of conservation. As an example, *S. supramontis* is a cave-dwelling species that is not encountered in numbers comparable to most pond-breeding amphibian species. Because it occurs on Sardinia and because infection is known to occur in plethodontids (Weinstein 2009), chytridiomycosis could pose a real threat to this

Table II. Current and ongoing studies of *Batrachochytrium dendrobatidis* (Bd) in Italy with information on species and study area.

Ongoing studies	Study area	Species	Source
Surveillance	Northwestern Piedmont	<i>Pelophylax</i> kl. <i>esculentus</i>	D. Seglie, S. Castellano, V. Botto, unpubl. data
Surveillance	Lombardy	<i>Bombina variegata</i>	A. Di Cerbo, pers. comm.
Surveillance	Liguria	<i>Bombina pachypus</i>	S. Canessa, F. Pasmans, A. Martel, F. Oneto, D. Ottonello, S. Salvidio, unpubl. data
Surveillance	Tuscany	<i>Bombina pachypus</i>	A. Crotini, B. Borri, G. Bruni, F. Giachi, pers. comm.
Preliminary surveillance	Lazio	<i>Pelophylax</i> kl. <i>hispanica</i>	Zirichiltaggi – SWC and Institute of Zoology, ZSL, pers. comm.
Surveillance on amphibian community	Central-southern Italy	All amphibian species, in particular <i>Bombina pachypus</i> , <i>Rana italica</i> and <i>Salamandra salamandra</i>	D. Canestrelli, M. Zampiglia, G. Nascetti, pers. comm.
Surveillance	Apennine area and Sardinia	Species of genus <i>Speleomantes</i>	F. Pasmans, pers. comm.
Broad-scale surveillance for Bd and fine-scale epidemiology at locations with infections	Sardinia	All amphibian species of the island, in particular <i>Discoglossus sardus</i> and <i>Euproctus platycephalus</i>	Zirichiltaggi – SWC, Ente Foreste delle Sardegna, University of Torino and Institute of Zoology, ZSL, pers. comm.
Preliminary surveillance	Sicily	<i>Pelophylax</i> kl. <i>hispanica</i> , <i>Xenopus laevis</i>	F. Andreone, pers. comm.

already endangered species. Caves may act as refuges for a proven host for *Bd*, *D. sardus* (Zirichiltaggi – SWC, unpubl. data). Field surveillance investigating the proximity of *D. sardus* populations to known *S. supramontis* populations, determining the prevalence of *Bd* in those frog populations and searching for frogs in caves where *S. supramontis* occurs could test this hypothesis. Additionally, these results would contribute to the growing database on *Bd* distribution in *D. sardus* populations on Sardinia. Similar approaches can test if populations of invasive hosts near threatened species include infected individuals, the relationship between fish stocking and infection and if other human activities are related to infection.

In the event infection is shown to be a risk, the question remains what approaches are appropriate for mitigating the effects of *Bd*. Existing treatments for infection all suffer from various shortcomings. Applying antifungals to wild populations is in all likelihood impossible, establishing antifungal bacteria stably in wild populations is problematic, and there are questions as to the appropriateness of introducing bacteria to counter the effects of a fungus. Elevated temperature may be as lethal to the amphibian host as to *Bd* but as with antifungals may also prove difficult to apply to a wild population (Woodhams et al. 2012). These types of mitigation are conceivably appropriate for control of disease in smaller populations and communities but it is unlikely they can be rolled out at a larger spatial scale (Garner et al. 2012). Instead, treatments may best be applied to captive populations and the study by Stagni et al. (2004) clearly illustrates the need for developing safe, effective treatments for species involved in captive programs intended to increase wild abundance (also see Walker et al. 2008). All such programs should include a comprehensive infection-testing scheme, including screens for *Bd*. If infection is detected, it is essential that any existing treatment be tested for each species before being applied. Effectiveness in one amphibian species is not evidence of effectiveness in another species and when applied inappropriately, exposure to treatments can cause serious tissue and organ damage, sterility and death (Garner et al. 2009a; Martel et al. 2011). Infection and disease monitoring and treatment in captive animals are the purview of veterinary science and are regulated at the national and European level. All captive conservation programmes must involve the input of certified veterinary surgeons, and treatment for infection with *Bd* requires consultation with veterinarians familiar with amphibian health and treatment of fungal diseases. *In situ* captive breeding centers can directly aid the study of *Bd* dynamics by providing animals for

experimental assessments of disease processes and anti-*Bd* treatments (e.g., Garner et al. 2009b).

But what of amphibians in the wild? Is it possible to mitigate *Bd* in wild amphibian populations and communities? If yes, this will not be accomplished without first having a sound understanding of what factors lead to the emergence of *Bd* in the community and how infection and disease are transmitted and maintained within it. Our increasing understanding of the epidemiology of *Bd* already has shown how one parameter, the proliferation of infection, can determine if a population will persist or suffer extirpation (Briggs et al. 2010). This finding suggests that by controlling factors that elevate the probability of proliferation (e.g., rate of intrusion of vectors, the reproductive rate of *Bd*, the density of highly susceptible hosts) it may be possible to reduce infection to a level where host populations are sustained. Infection elimination may be impossible, but as much of medical history has shown, epidemics that cannot be prevented can be managed.

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References

- Adams MJ, Galvan S, Scalera R, Grieco C, Sindaco R. 2008. *Batrachochytrium dendrobatidis* in amphibian populations in Italy. *Herpetological Review* 39:324–326.
- Alford RA, Richards SJ. 1999. Global amphibian declines: A problem in applied ecology. *Annual Review of Ecology, Evolution, and Systematics* 30:133–165.
- Andreone F, Corti C, Sindaco R, Romano A, Giachi A, Vanni S, Delfino G. 2009a. *Bombina pachypus*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. Available: www.iucnredlist.org. Accessed Nov 2012 22.
- Andreone F, Lecis R, Edgar P, Corti C, Sindaco R, Romano A. 2009b. *Speleomantes supramontis*. In: IUCN 2012. IUCN Red

- List of Threatened Species. Version 2012.2. Available: www.iucnredlist.org. Accessed Nov 2012 22.
- Andreone F, Sindaco R. 1998. Erpetologia del Piemonte e della Valle d'Aosta, Atlante degli Anfibi e dei Rettili, Monografie XXVI (1998). Torino: Museo Regionale di Scienze Naturali.
- Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips KR, Marantelli G, Parkes H. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. *Proceedings of the National Academy of Sciences USA* 95:9031–9036.
- Bielby J, Bovero S, Sotgiu G., Tessa G., Favelli M, Angelini C, Doglio S, Clare FC, Gazzaniga E, Lapietra F, Garner TWJ. 2009. Fatal chytridiomycosis in the Tyrrhenian painted frog. *EcoHealth* 6:27–32.
- Bielby J, Cooper N, Cunningham AA, Garner TWJ, Purvis A. 2008. Predictors of rapid decline in frog species. *Conservation Letters* 1:82–90.
- Blaustein AR, Belden LK, Olson DH, Green DL, Root TL, Kiesecker JM. 2001. Amphibian breeding and climate change. *Conservation Biology* 15:1804–1809.
- Blaustein AR, Romansic JM, Kiesecker JM, Hatch AC. 2003. Ultraviolet radiation, toxic chemicals and amphibian population declines. *Diversity and Distributions* 9:123–140.
- Bosch J, Carrascal L M, Durán L, Walker S, Fisher MC. 2007. Climate change and outbreaks of amphibian chytridiomycosis in a montane area of Central Spain; is there a link? *Proceedings of the Royal Society of London B* 274:253–260.
- Bosch J, Martínez-Solano I. 2006. Chytrid fungus infection related to unusual mortalities of *Salamandra salamandra* and *Bufo bufo* in the Peñalara Natural Park, Spain. *Oryx* 40: 84–89.
- Bosch J, Martínez-Solano I, García-Paris M. 2001. Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas in central Spain. *Biological Conservation* 97:331–337.
- Bovero S, Bielby J, Garner TWJ, Angelini C, Doglio S, Favelli M, Gazzaniga E, Sotgiu G, Tessa G. 2008a. The emergence of *Batrachochytrium dendrobatidis* in Sardinia. In: Corti C, editor. *Herpetologia Sardiniae. "le scienze"* (8). Latina: Societas Herpetologica Italica/Edizioni Belvedere. pp. 90–92.
- Bovero S, Sotgiu G, Angelini C, Doglio S, Gazzaniga E, Cunningham AA, Garner TWJ. 2008b. Detection of Chytridiomycosis caused by *Batrachochytrium dendrobatidis* in the endangered Sardinian newt (*Euproctus platycephalus*) in southern Sardinia, Italy. *Journal of Wildlife Diseases* 44:712–715.
- Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms* 60:141–148.
- Briggs CJ, Knapp RA, Vredenburg VT. 2010. Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proceedings of the National Academy of Sciences USA* 107:9695–9700.
- Carey C, Cohen N, Rollins-Smith L. 1999. Amphibian declines: An immunological perspective. *Developmental & Comparative Immunology* 23:459–472.
- Carletti S, Spilinga C. 2006. Gli Anfibi e i Rettili della Zona di Protezione Speciale "Lago Trasimeno" (IT 5210070). In: Abstracts VI Congresso Societas Herpetologica Italica (S.H.I.), 27 settembre–1 ottobre 2006, Roma.
- Cushman SA. 2006. Effects of habitat loss and fragmentation on amphibians: A review and prospectus. *Biological Conservation* 128:231–240.
- Daszak P, Berger L, Cunningham AA, Hyatt AD, Green GE, Speare R. 1999. Emerging infectious diseases and amphibian population decline. *Emerging Infectious Diseases* 5:735–748.
- Daszak P, Cunningham AA, Hyatt A.D. 2000. Emerging infectious diseases of wildlife—Threats to biodiversity and human health. *Science* 287:443–449.
- Daszak P, Cunningham AA, Hyatt AD. 2003. Infectious disease and amphibian population declines. *Diversity and Distributions* 9:141–150.
- Densmore CL, Green DE. 2007. Diseases of amphibians. *Ilar Journal* 48:235–254.
- Duffus ALJ, Cunningham AA. 2010. Major disease threats to European amphibians. *Herpetological Journal* 20:117–127.
- Farrer RA, Weinert LA, Bielby J, Garner TWJ, Balloux F, Clare F, Bosch J, Cunningham AA, Weldon C, du Preez LH, Anderson L, Kosakovsky Pond SL, Shahar-Golan R, Henk DA, Fisher MC. 2011. Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. *Proceedings of the National Academy of Sciences USA* 108:18732–18736.
- Federici S, Clemenzi S, Favelli M, Tessa G, Andreone F, Casiraghi M, Crottini A. 2008. Identification of the pathogen *Batrachochytrium dendrobatidis* in amphibian populations of a plain area in the Northwest of Italy. *Herpetology Notes* 1:33–37.
- Ficetola FG, Scali S. 2010. Invasive amphibians and reptiles in Italy In: Di Tizio L, Di Cerbo AR, Di Francesco N, Cameli A, editors. *Programma e Riassunti VIII Congresso Nazionale Societas Herpetologica Italica* (Chieti, 22–26 settembre 2010). Chieti: Tipografia Brandolini. pp. 20–21.
- Ficetola FG, Valentini A, Miaud C, Noferini A, Mazzotti S, Dejean T. 2011. *Batrachochytrium dendrobatidis* in amphibians from the Po River Delta, Northern Italy. *Acta Herpetologica* 6:297–302.
- Fisher MC. 2009. Endemic and introduced haplotypes of *Batrachochytrium dendrobatidis* in Japanese amphibians: Sink or source? *Molecular Ecology* 18:4731–4733.
- Fisher MC, Bosch J, Yin Z, Stead DA, Walker J, Selway L, Brown AJP, Walker LA, Gow NAR, Stajich JE, Garner TWJ. 2009a. Proteomic and phenotypic profiling of the amphibian pathogen *Batrachochytrium dendrobatidis* shows that genotype is linked to virulence. *Molecular Ecology* 18:415–429.
- Fisher MC, Garner TWJ. 2007. The relationship between the introduction of *Batrachochytrium dendrobatidis*, the international trade in amphibians and introduced amphibian species. *Fungal Biology Reviews* 21:2–9.
- Fisher MC, Garner TWJ, Walker SF. 2009b. The global emergence of *Batrachochytrium dendrobatidis* in space, time and host. *Annual Review of Microbiology* 63:291–310.
- Garner TWJ, Briggs CJ, Bielby J, Fisher MC. 2012. Determining when parasites of amphibians are conservation threats to their hosts: Methods and perspectives. In: Aguirre AA, Ostfeld R, Daszak P, editors. *Conservation medicine: Applied cases of ecological health*. Oxford, NY: Oxford University Press. pp. 521–538.
- Garner TWJ, Garcia G, Carroll B, Fisher MC. 2009a. Using itraconazole to clear *Batrachochytrium dendrobatidis* infection and subsequent depigmentation of *Alytes muletensis* tadpoles. *Diseases of Aquatic Organisms* 83:257–260.
- Garner TWJ, Pearman PB, Cunningham AA, Fisher MC. 2004. Population genetics and disease threats across the entire range of *Rana latastei*. A paper presented at this conference.

V° congresso Nazionale della Societas Herpetologica Italica, 29 September–3 October 2004, Calci (Pisa), p. 62.

Garner TWJ, Perkins MW, Govindarajulu P, Seglie D, Walker S, Cunningham AA, Fisher MC 2006. The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana*. *Biology Letters* 2:455–459.

Garner TWJ, Walker S, Bosch J, Hyatt AD, Cunningham AA, Fisher MC. 2005. Chytrid fungus in Europe. *Emerging Infectious Diseases* 11:1639–1641.

Garner TWJ, Walker S, Bosch J, Leech S, Rowcliffe JM, Cunningham AA, Fisher MC. 2009b. Life history trade-offs influence mortality associated with the amphibian pathogen *Batrachochytrium dendrobatidis*. *Oikos* 118:783–791.

Goka K, Yokoyama J, Une Y, Kuroki T, Suzuki K, Nakahara M, Kobayashi A, Inaba S, Mizutani T, Hyatt AD. 2009. Amphibian chytridiomycosis in Japan: Distribution, haplotypes and possible route of entry into Japan. *Molecular Ecology* 18:4757–4774.

Hyatt AD, Boyle DG, Olsen V, Boyle DB, Berger L, Obendorf D, Dalton A, Kriger K, Hero M, Hines H, Phillot R, Cambell R, Marantelli G, Gleason F, Colling A. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 73:175–192.

James TY, Livintseva AP, Vilgalys R, Morgan JAT, Taylor JW, Fisher MC, Berger L, Weldon C, du Preez L, Longcore JE. 2009. Rapid global expansion of the fungal disease chytridiomycosis into declining and healthy amphibian populations. *PLoS Pathogens* 5:e1000458. doi:10.1371/journal.ppat.1000458

Johnson ML, Speare R. 2005. Possible modes of dissemination of the amphibian chytrid *Batrachochytrium dendrobatidis* in the environment. *Diseases of Aquatic Organisms* 65:181–186.

Kats LB, Ferrer RP. 2003. Alien predators and amphibian declines: Review of two decades of science and the transition to conservation. *Diversity and Distributions* 9:99–110.

Kruger KM, Hero JM. 2007. Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. *Journal of Zoology* 3:352–359.

Lanza B. 1983. Anfibi, rettili [Amphibia, Reptilia]. Guide per il riconoscimento delle specie animali delle acque interne italiane. In: Ruffo S, editor. Collana del progetto finalizzato 'Promozione della qualità dell'ambiente', Verona: C.N.R. p. 27.

Lanza B, Ferri V. 1997. *Rana catesbeiana* Shaw, 1802. In: Gasc J-et-al, editors. Atlas of amphibians and reptiles in Europe. Paris: SEH and Museum National d'Histoire Naturelle (IEGB/SPN). pp. 132–133.

Li Vigni F, Licata F, Anzà S. 2011. Waterfrog (*Pelophylax* sp.) found near Domusnovas in southwestern Sardinia, Italy. *Herpetozoa* 24:101–103.

Lillo F, Marrone F, Sicilia A, Castelli G, Zava B. 2005. An invasive population of *Xenopus laevis* (Daudin, 1802) in Italy. *Herpetozoa* 18:63–64.

Lips KR, Brem F, Brenes R, Reeve JD, Alford RA, Voyles J, Carey C, Livo L, Pessier AP, Collins JP. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences USA* 103:3165–3170.

Lips KR, Diffendorfer J, Mendelson III JR, Sears MW. 2008. Riding the wave: Reconciling the roles of disease and climate change in amphibian declines. *PLoS Biology* 6:e72. doi:10.1371/journal.pbio.0060072

Longcore JE, Pessier AP, Nichols DK. 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* 91:219–227.

Martel A, Van Rooij P, Vercauteren G, Baert K, Van Waeyenberghe L, Debacker P, Garner TWJ, Ducatelle R, Haesebrouck F, Pasmans F. 2011. Developing a safe antifungal treatment protocol to eliminate *Batrachochytrium dendrobatidis* from amphibians. *Medical Mycology* 49:143–149.

Mazzoni R, Cunningham AA, Daszak P, Apolo A, Perdomo E, and Speranza G. 2003. Emerging pathogen of wild amphibians in frogs (*Rana catesbeiana*) farmed for international trade. *Emerging Infectious Diseases* 9:995–998.

Mazzotti S, Caramori G, Barbieri C. 1999. Atlante degli Anfibi e dei Rettili dell'Emilia-Romagna (aggiornamento 1993/1997). Quaderni della Stazione Ecologica del Museo Civico di Storia Naturale di Ferrara 12:1–121.

Mazzotti S, Mantovani S, Penazzi R, Cavaliere d'Oro A, Gentile V, Rossini M, Lizzio L, Rizzati E, Frasson F, Mingozzi V, Noferini A. 2007. La comunità degli Anfibi del Parco del Delta del Po. Quaderni della Stazione di Ecologia del Civico Museo di Storia Naturale di Ferrara 17:49–58.

Mutschmann F, Berger L, Zwart P, Gaedicke C. 2000. Chytridiomycosis in amphibians: First report in Europe. *Berl Muench Tieraerztl Wochenschr* 113:380–383.

Ohst T, Graser Y, Mutschmann F, Plötner J. 2011. Neue Erkenntnisse zur Gefährdung europäischer Amphibien durch den Hautpilz *Batrachochytrium dendrobatidis*. *Zeitschrift für Feldherpetologie* 18:1–17.

OIE (World Organisation of Animal Health). 2008. OIE Report of the meeting of the OIE Aquatic Animal Health Standards Commission. Paris, 3–7 March 2008, 76th General Session. Available: [www.oie.int/aac/eng/FDC%20reports/Mar%202008%20report%20\(English\).pdf](http://www.oie.int/aac/eng/FDC%20reports/Mar%202008%20report%20(English).pdf)

Orrù F, Deiana AM, Cau A. 2010. Introduction and distribution of alien freshwater fishes on the island of Sardinia (Italy): An assessment on the basis of existing data sources. *Journal of Applied Ichthyology* 26(Suppl. 2):46–52.

Pasmans F, Muijsers M, Maes S, Van Rooij P, Brutyn M, Ducatelle R, Haesebrouck F, Martel A. 2010. Chytridiomycosis related mortality in a Midwife toad (*Alytes obstetricans*) in Belgium. *Vlaams Diergeneeskundig Tijdschrift* 79:461–463.

Pritchard PCH. 1979. Head-starting and other conservation techniques for marine turtles Cheloniidae and Dermochelyidae. *International Zoo Yearbook* 19:38–42.

Rachowicz LJ, Hero JM, Alford RA, Taylor JW, Morgan JAT, Vredenburg VT, Collins JP, Griggs CJ. 2005. The novel and endemic pathogen hypotheses: Competing explanations for the origin of emerging infectious diseases of wildlife. *Conservation Biology* 19:1441–1448.

Rachowicz LJ, Knapp RA, Morgan JAT, Stice MJ, Vredenburg VT, Parker JM, Briggs CJ. 2006. Emerging infectious disease as a proximate cause of amphibian mass mortality. *Ecology* 87:1671–1683.

Romano A, Sindaco R, Andreone F, Lecis R, Edgar P, Schmidt B, Corti C. 2009. *Euproctus platycephalus*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. Available: www.iucnredlist.org. Accessed Nov 2012 22.

Schlaepfer MA, Hoover C, Dodd, CK. 2005. Challenges in evaluating the impact of the trade in amphibians and reptiles on wild populations. *Bioscience* 55:256–264.

Scott C, Bell BD, Waldman B. 2010. Does chytridiomycosis disrupt amphibian skin function? *Copeia* 3:487–495.

Simoncelli F, Fagotti A, Dall'Olio R, Vagnetti D, Pascolini R, Di Rosa I. 2005. Evidence of *Batrachochytrium dendrobatidis* infection in water frogs of the *Rana esculenta* complex in Central Italy. *Ecohealth* 2:307–312.

Skerratt LF, Berger L, Spear R, Cashins S, McDonald KR, Phillott AD, Hines HB, Kenyon N. 2007. Spread of

- chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth* 4:125–134.
- 780 Stagni G, Dall'Olio R, Fusini U, Mazzotti S, Scoccianti C, Serra A. 2004. Declining populations of Apennine yellow-bellied toad *Bombina pachypus* in northern Apennines, Italy: Is *Batrachochytrium dendrobatidis* the main cause? *Italian Journal of Zoology* (Suppl. 2):151–154.
- 785 Stagni G, Scoccianti C, Fusini R. 2002. Segnalazione di chytridiomicosi in popolazioni di *Bombina pachypus* (Anura, Bombinatoridae) dell'Appennino toscoemiliano. A paper presented at this conference. IV Congresso della Societas Herpetologica Italica. Societas Herpetologica Italica, Napoli.
- 790 Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller RW. 2004. *Science* 306: 1783–1786.
- Sztatecsny M, Glaser F. 2011. From the eastern lowlands to the western mountains: First records of the chytrid fungus *Batrachochytrium dendrobatidis* in wild amphibian populations from Austria. *Herpetological Journal* 21:87–90.
- 795 Tobler U, Schmidt BR. 2010. Within- and among-population variation in chytridiomycosis-induced mortality in the toad *Alytes obstetricans*. *PLoS ONE* 5:e10927.
- 800 Voyles J, Berger L, Young S, Speare R, Webb R, Warner J, Rudd D, Campbell R, Skerratt LF. 2007. Electrolyte depletion and osmotic imbalance in amphibians with chytridiomycosis. *Diseases of Aquatic Organisms* 77:113–118.
- Vredenburg VT, Knapp RA, Tunstall TS, Briggs CJ. 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. *Proceedings of the National Academy of Sciences USA* 107: 9689–9694. 805
- Walker SF, Bosch J, Gomez V, Garner TWJ, Cunningham AA, Schmeller DS, Ninyerola M, Henk D, Ginestet C, Arthur CP, Fisher MC. 2010. Factors driving pathogenicity vs. prevalence of amphibian panzootic chytridiomycosis in Iberia. *Ecology Letters* 13:372–382. 810
- Walker SF, Bosch J, James TY, Litvintseva AP, Valls JAO, Piña S, Garcia G, Rosa GA, Cunningham AA, Hole S, Griffiths RA, Fisher MC. 2008. Invasive pathogens threaten species recovery programs. *Current Biology* 18:R853–R854. 815
- Weinstein SB. 2009. An aquatic disease on a terrestrial Salamander: Individual and population level effects of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, on *Batrachoseps attenuatus* (Plethodontidae). *Copeia* 4:653–660. 820
- Weldon C, du Preez LH, Hyatt AD, Muller R, Speare R. 2004. Origin of the amphibian chytrid fungus. *Emerging Infectious Diseases* 10:2100–2105.
- Woodhams DC, Geiger CC, Reinert LK, Rollins-Smith LA, Lam B, Harris RN, Briggs CJ, Vredenburg VT, Voyles J. 2012. Treatment of amphibians infected with chytrid fungus: Learning from failed trials with itraconazole, antimicrobial peptides, bacteria, and heat therapy. *Diseases of Aquatic Organisms* 98:11–25. 825